Example 200

Efficacy of Immunomodulatory Compounds In Ebola Challenge Model

[1000] Additional testing in an Ebola Challenge Model was conducted with the immunomodulatory agents CpG (ODN-1826), poly I:C, LPS, Pam2CSK4 and Pam3CSK4 (synthetic triacylated lipopeptides), and R-848 (resiquimod). The compounds were administered to eight to twelve-week old C57BL/6 mice by intraperitoneal (IP) or intramuscular (IM) injection. For IP injection, compounds were administered 2 h prior to IP injection of EBOV, and then again on days 2, 4, 6 and 8 following EBOV challenge. For IM injection, compounds were administered 2 hours prior to IP injection of EBOV. Efficacy of the immunomodulatory compounds compared to vehicle-control treatment was assessed on 14-day survival.

[1001] Survival of mice was monitored and is reported in the tables below.

TABLE 6

Efficacy of Immunomodulatory Agents Against Ebola Virus in Mice

	Target Receptor	IP	IM
CpG (ODN-1826)	TLR9	X	X
Poly I:C	TLR3, RIGI, others	✓	X
LPS	TLR4	X	X
Pam2CSK4	TLR 2/6	NT	X
Pam3CSK4	TLR1/2	X	X
R-848	TLR7/8	X	X

X = agent showed no efficacy
✓ = agent showed efficacy
NT = Not tested.

Example 201

Benzonapthyridine SMIPs Administered Intraperitoneally and Intramuscularly in Guinea Pigs Induced an Immune Response

[1002] Guinea pigs were given SMIPs of the invention by intraperitoneal (IP) or intramuscular (IM) injection 2 hours prior to subcutaneous injection of guinea pig-adapted Ebola virus. On day 0 (zero), 2 hours after the administration of compounds, each guinea pig was administered a subcutaneous injection of 1,000 PFU of guinea pig-adapted Ebola virus. Guinea pigs (6 guinea pigs per group) were administered, vehicle (peanut oil) alone, PolyI:C (100 μg), R-848 (100 μg), or SMIP 28 (100 μg or 10 μg). The guinea pigs were given additional daily IP injections of SMIP, vehicle alone, Poly I:C, or R-848 on days 1, 2, 3, 4, 6, 8, and 10 post-infection with guinea pig-adapted Ebola virus. Survival of guinea pigs was monitored on a daily basis for sixteen days following initial treatment. In addition to survival, weight gain or loss, and individual guinea pigs were given clinical scores based on cage-side observations. The clinical scores were determined according to the criteria provided in Table 7.

TABLE 7

Clinical Score	Clinical Observations
0	Healthy; no clinical signs of disease, animal active and responsive
1	Slightly ruffled fur, reduced mobility
2	Severely reduced mobility, hunched posture, ruffled fur, reduced responsiveness
3	Moribund; Unresponsive, non-mobile, labored breathing
4	Dead

[1003] Results of the study are presented in FIGS. **3**, **4**A, and **4**B.

[1004] As shown in FIG. **3**, IP or IM SMIP 28 prolonged survival and resulted in an overall increase in survival. SMIP 28 at 100 μg delayed mortality and resulted in increased survival in comparison to R-848 and Poly I:C.

[1005] FIG. **4**A provides the guinea pigs' weights, as a percent of the starting weights, over the course of the experiment. The results show that guinea pigs to which 100 μg of R-848 or Poly I:C was administered intraperitoneally experienced a steady increase in weight. Also, guinea pigs to which 100 μg or 10 μg SMIP 28 was administered intraperitoneally experienced a steady increase in weight. The weight gain in the SMIP 28-treated guinea pigs was greater than those treated with R-848 or Poly I:C. The weight gain results correlate with the delayed mortality and increased survival rate of guinea pigs treated with SMIP 28 at 100 μg compared to those treated with 100 μg R-848 or Poly I:C.

[1006] FIG. **4**B provides the clinical scores given to the guinea pigs of this experiment. The results show that SMIP 28-treated guinea pigs had less severe symptoms than untreated animals, and that the group that received 100 μg SMIP 28 IP had a marked delay in onset and decrease in severity of symptoms.

[1007] The data demonstrate that SMIP 28 administered intraperitoneally and intramuscularly is capable of protecting guinea pigs challenged with guinea pig-adapted Ebola virus. Additionally, the data demonstrate that SMIP 28 outperformed the anti-viral efficacy of R-848 and Poly I:C.

Example 202

Benzonapthyridine SMIPs Administered Intraperitoneally and Intramuscularly in Guinea Pigs Challenged Intraperitoneally and Subcutaneously with Guinea Pig-Adapted Ebola Virus Induced an Immune Response

[1008] Guinea pigs were given SMIPs of the invention by intraperitoneal (IP) or intramuscular (IM) injection 2 hours prior to IP or subcutaneous injection of guinea pig-adapted Ebola virus. On day 0 (zero), 2 hours after the administration of compounds, each guinea pig was administered an IP or subcutaneous injection of 1,000 PFU of guinea pig-adapted Ebola virus. Guinea pigs were administered, vehicle alone, PolyI:C (100 μg), R-848 (100 μg), or SMIP 28 (100 μg or 10 μg), each in vehicle. The guinea pigs were given additional daily IP injections of SMIP, vehicle alone, Poly I:C, or R-848 on days 1, 2, 3, 4, 6, 8, and 10 post-infection with guinea pig-adapted Ebola virus. Survival of guinea pigs was monitored on a daily basis for sixteen days following initial treatment.

[1009] Results of the study are presented in FIGS. **5**A and **5**B.